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Serum Level of Ligand Programmed Death-1 in Endometriosis

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Abstract

AIM: In this study, is to evaluate serum level of ligand programmed death-1 (PDL1) in patients with genital endometriosis.

MATERIAL AND METHODS: For PDL-1, cancer antigen 125 (CA 125), interleukin (IL)-6, IL-8, tumor necrosis factor (TNF), and vascular endothelial growth factor (VEGF) determination, venous blood was taken 1 h before surgery and/or treatment. All patients were stratified by the presence or absence of endometriosis according to the American Society of Reproductive Medicine's Revised Classification of Endometriosis of the American Society of Reproductive Medicine.

RESULTS: Twenty-one patients with endometriosis participated in the study. The PDL-1 level an experienced group was 55.32 ng/ml (interquartile range [IQR] 34.53–76.20); in the control group was 19.72 ng/ml (IQR 14.72–24.78), which was a statistically significant decrease ($p < 0.001$). A significant increase in CA125 31.87 (IQR 15.43–36.96) was detected ($p < 0.001$). In the experimental group, all pro-inflammatory cytokines were elevated (IL-6, IL-8, and TNF, $p < 0.013$; <0.001; and <0.001) and almost twice increased VEGF 243.44 (IQR 194.56–328.07), ($p = 0.016$). A noticeable correlation was found between the following indicators PDL-1-CA125, PDL-1-IL-8, and PDL-1-TNF, and a moderate correlation was found between PDL-1 and VEFF ($p = 0.006$).

CONCLUSIONS: The results of the study showed that the levels of PDL-1, CA 125, IL-6, IL-8, TNF, and VEGF were statistically significantly different in the experimental and control groups, and a correlation was also revealed between the levels of PDL-1 and CA125, IL-8 and TNF in patients with genital endometriosis. Therefore, further studies with larger numbers of patients are required.

Introduction

Endometriosis is a chronic disease characterized by endometrial tissues located outside the uterine cavity. This disease affects about 10–15% of women of reproductive age and up to 20–30% of infertile women [1]. Clinically, endometriosis is mainly manifested by pelvic pain, dysmenorrhea, dyspareunia, and infertility. Surgical and/or hormone therapy remains the primary therapy of choice. However, these treatments are associated with side effects and recurrence of endometriosis in 30–50% of women within 3–5 years after surgery [2].

At present, there are many etiological theories why endometriosis occurs, yet none of them explains all the existing cases of this disease. Endometriosis can only be diagnosed by invasive manipulations to confirm its morphology as there are no specific biomarkers in the blood, saliva, or urine that could be used to diagnose and select proper treatment. Therefore, the study of the pathogenesis of endometriosis is still an urgent problem in the modern world. The study of the immune aspects related to the development of endometriosis is one of the promising areas of recent times, since the study of the relationship between endometriosis and the immune system revealed some changes in the cellular immunity system: Impaired function of T-cells and B-cells, natural killer (NK) cells, changes in interleukin (IL) levels, tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF) [3]. These data can be used both to study the fundamental mechanisms of development and to try to use them for diagnostic procedures.

The programmed death 1 protein (PD-1) is an immune checkpoint receptor that provides immunosuppression. PD-1 prevents autoimmunity, controls damage to healthy tissues during infection. PD-1 is known to be expressed in NK cells, B-cells, dendritic cells, antigen-presenting cells, activated CD4+ and CD8+ T-cells, and monocytes [4], [5]. In its turn, PD-1 binds to one of its ligands, PD-L1 or PD-L2, thereby inhibiting the activation of T-cells [6]. The high expression of PD-1 on the surface of T-cells alters its ability to eliminate cancer and infectious diseases, on the basis

of which many PD-1 inhibitors have been developed to treat these diseases [7], [8]. PD-1 molecules can be expressed not only in the immunocompetent cells but also in tissue cells, including tumors. Their interaction leads to the development of immune system tolerance and activation or deactivation of the immune response to this antigen.

This mechanism may bear a relation to endometriosis. Getting into the abdominal cavity, endometrial cells provoke an influx of leukocytes to the site of their attachment, which activates local immunity. Macrophages are involved in the recognition of foreign and damaged cells in the abdominal cavity. Further on, the synthesis of a chain of ILs, such as TNF-α and IL-4, commences, which signals about the acute phase of the process. TNF-α mainly acts as a precursor to initiate an inflammatory response in the acute phase by activating a cascade of other cytokines such as IL-1, IL-6, and VEGF. In addition, it may promote the adhesion of ectopic endometrial cells to the peritoneum [9], which may be associated with impaired function of apoptosis cells, which are regulated by sFas, FasL, and other molecules.

Taking into account the above data, we assume that an increase in the level of programmed cell death proteins in serum will give grounds to assume its possible role in the pathogenesis of endometriosis and regulation of the immune system response to ectopic foci. And in the future, PD-1/PD-L1 inhibitors could be used as a therapeutic strategy for endometriosis. In this regard, the aim of this study is to evaluate serum level of ligand PD-1 (PD L1) in patients with genital endometriosis.

Materials and Methods

This study involved 40 females at the age of 18 and over. The study was authorized by the Bioethics Committee of the KMU under No. 10 dated March 16, 2020. In all the cases, a voluntary informed consent was obtained to participate in the study. The 1st (control) group was represented by 19 conditionally healthy individuals. The 2nd (experimental) group included 21 patients with initially diagnosed genital endometriosis.

The criteria for the participants to be included into an experimental group were as follows: Patients with genital endometriosis over 18 years of age who have received no previous treatment for this disease. The exclusion criteria in both groups were as follows: Age under 18 years, pregnant and lactating women, patients with autoimmune and oncological diseases, patients with HIV infection, patients taking medication that may affect the immune system or hormonal drugs, as well as patients with an ongoing infectious process that occurred at least 4 weeks before the study.

Blood was sampled in the morning, on an empty stomach, before the start of treatment and/or surgery. To determine the test markers in the blood serum, an immunofluorescent method of multiplex determination was used with the magnetic spheres and the following analytes: sFas, FasL, VEGF, ILs (IL-6, IL-8, and IL-18), CA125, HE 4, and leptin using the Human Circulation Biomarker panel of the Milliplex Map (Millipore) series. The PD-L1 marker was determined by the immunofluorescence method using the Human ProcartaPlex™ Kit (Thermo Fisher) using XMap technology.

Statistical analysis was carried out using the StarTech v.2.4. 5 software (developed by StarTech LLC, Russia). Quantitative indicators were evaluated for the compliance with the normal distribution using the Shapiro–Wilk criterion (with the number of subjects <50). Given the lack of a normal distribution, the quantitative data were interpreted using the median (Me) as well as the lower and upper quartiles (Q1–Q3). The comparison of the two groups against a quantitative indicator, the distribution of which differed from the normal one, was performed by the Mann–Whitney U-criterion. The comparison of percentages in the analysis of multipole conjugation tables was performed using the Pearson's Chi-squared test criterion. The direction and closeness of the correlation relationship between the two quantitative indicators were estimated using the Spearman rank correlation coefficient. The predictive model characterizing the dependence of the quantitative variables on the factors was developed using a linear regression method.

Results

The detailed information about patients is presented in Table 1. This pilot study included 40 participants divided into two groups: Control (average age of 30 [28; 40]) and experimental (average age of 36.0 [30; 43]). When comparing weight, height, menarche, and first sex between the two groups, there were no statistically significant differences.

Table 1: Descriptive statistics of the studied groups

Indicators	Groups	Me	Q_1–Q_3	n	Min.	Max.
Age	Control group, (years)	30.0	28.0–40.0	19	21.0	43.0
	Experimental group, (years)	36.0	30.0–43.0	21	22.0	53.0
Weight	Control group, (kg)	56.0	53.5–64.0	19	47.0	110.0
	Experimental group, (kg)	64.0	60.0–74.0	21	50.0	98.0
Growth	Control group, (cm)	1.6	1.6–1.7	19	1.5	1.8
	Experimental group, (cm)	1.6	1.6–1.6	21	1.5	1.8
IMT	Control group	21.8	20.7–24.3	19	17.7	35.5
	Pilot group	23.8	21.8–27.0	21	19.3	46.0
Menarche	Control group, (years)	12.0	12.0–13.0	19	11.0	14.0
	Experimental group, (years)	12.0	11.0-13.0	21	10.0	16.0
Age at first sex	Control group, (years)	19.0	7.5–22.0	19	0.0	25.0
	Experimental group, (years)	19.0	17.0–20.0	21	0.0	29.0

In accordance with the given study, 10 out of 21 patients were diagnosed with adenomyosis (47.6%) as per ICD 10 and 11 of them with endometriosis of the ovaries (external endometriosis) (52.4%),

(endometriosis according to ICD 10). Furthermore, the stages of these diseases were determined according to the r-AFS classification (American Society for Reproductive Medicine, Revised American Society for Reproductive Medicine Classification of endometriosis, 1997). The distribution by stages in the two ICD diseases was as follows: Stage I – 5 (23.8%), Stage II – 14 (66.7%), and Stage III – 2 (9.5%). According to the purpose of the study, the studied diagnostic markers in both groups were analyzed.

We found a significant increase in the CA125 tumor marker ($p < 0.001$). In the experimental group, the following pro-inflammatory cytokines IL-6, IL-8 and TNF were elevated ($p < 0.013$; <0.001; <0.001) compared to the control group. It is worth mentioning that PDL-1 serum levels in the group of women with endometriosis were significantly high ($p < 0.001$) with the VEGF level ($p = 0.016$) almost doubled. Taking into account, the statistically significant difference in the following indicators: PDL-1, CA 125, IL-6, IL-8, TNF, and VEGF, an analysis of their dependence on the studied diseases, was carried out as per the endometriosis according to ICD 10.

According to the table presented above, when comparing the levels of markers, only the CA 125 level was found to have a statistically significant difference depending on endometriosis ICD 10 ($p = 0.029$). Given the statistical differences in the PDL-1 level, this study included a correlation analysis of the relationship between the PDL-1 score and other diagnostic markers.

The results of the correlation analysis of the relationship between the PDL-1 and other diagnostic markers under the study are graphically demonstrated in Figure 1. It is obvious that there is no statistically significant relationship between PDL-1–IL-18 and PDL-1–HE 4. A significant relationship can be seen between the following indicators PDL-1–CA125, PDL-1–IL-8, and PDL-1–TNF ($p < 0.001$, <0.001*, and <0.001*, respectively). The observed dependence of CA 125 on PDL-1 can be explained by the equation of paired linear regression: $Y_{CA125} = 0.362 \times X_{PDL-1} + 4.548$. This means that with an increase in PDL-1 by 1 pg/ml, one should expect an increase in CA 125 by 0.362 U/ml. The resulting model explains the 20.8% of the observed variance of CA125.

The dependence of IL-8 on the PDL-1 is explained by the equation of paired linear regression: $Y_{IL-8} = 0.424 \times X_{PDL-1} + 5.178$. According to this equation, if the PDL-1 indicator increases by 1 pg/ml, one should expect an increase in the IL-8 level by 0.424. The resulting model explains the 6.1% of the observed variance of the IL-8 indicator. The observed dependence of the TNF indicator on the PDL-1 level is expressed by the paired linear regression equation: $Y_{TNF} = 0.297 \times X_{PDL-1} + 9.533$.

Therefore, with an increase in the level of PDL-1 by 1 pg/ml, one should see an increase in TNF by 0.297 pg/ml.

Discussion

The PD-1/PD-L1 system plays a crucial role in immune responses and peripheral tolerance. In pathological conditions, this pathway can suppress the protective reactions of T-cells and cause immune system disorders. Endometriosis is known to be an estrogen-dependent and immune-related disease. The expression of PD-1 and PD-L1 has been studied in various cancer and autoimmune diseases [5]. However, several studies have attempted to examine their expression and the possibility of their regulation by estrogen in endometriosis. In the study conducted by Wu *et al.*, the PD-1/PD-L1 expression was observed in both eutopic and ectopic endometrium. Their expression was also higher in patients with endometriosis. In addition, when treated with 17β-estradiol, it enhanced PD-L1 expression in eutopic epithelial cells in endometriosis. These findings suggest that the PD-1/PD-L1 pathway may be involved in the immune dysfunction of endometriosis and regulated by estrogen [10]. In the given study, the obtained data suggest that the PDL-1 serum levels are clearly increased in endometriosis ($p < 0.001$*) and can be considered as a new potential diagnostic marker. Although in determining the level of PDL-1 in uterine adenomyosis and endometriosis of the ovaries, statistically significant results were not obtained ($p = 0.455$).

The CA125 remains the main diagnostic marker to determine an ovarian cancer, but in recent years, there have been studies that confirm the role of CA125 in endometriosis [11], [12]. Karimi-Zarchi *et al.* found a significant correlation between the stage of endometriosis, the size of the lesion, the assessment of adhesion, and the pre-operative concentration of CA125 in the blood plasma [13]. Our data showed the significance of the CA125 level in endometriosis – 31.87 [15,43; 36,96] (Me [Q1; Q3]), as well as at comparison of adenomyosis and endometriosis of the ovaries ($p = 0.032$). When assessing the correlation relationship between PDL-1 and CA125, statistically significant results were found. However, given the insufficient research, it is necessary to continue the study with a larger range of sampling. Mihalyi *et al.* found that plasma levels of IL-6, IL-8, and CA-125 were elevated in all women with endometriosis and in women with lower mild endometriosis with 87% of sensitivity and 71% of specificity [14]. In the given study, the IL-6 and IL-8 ILs were statistically significantly different from the control group ($p = 0.013$ and <0.001, respectively). When conducting a systematic review of inflammatory mediators in endometriosis, Machairiotis *et al.* assessed the importance of the IL-6, IL-8, ILs, and their role in increasing pain in endometriosis. Given the findings on the inhibition of inflammatory mediators as a method of treating pain, it is concluded that there is a promising therapy that is an alternative to hormone-dependent drugs widely used these days [15].

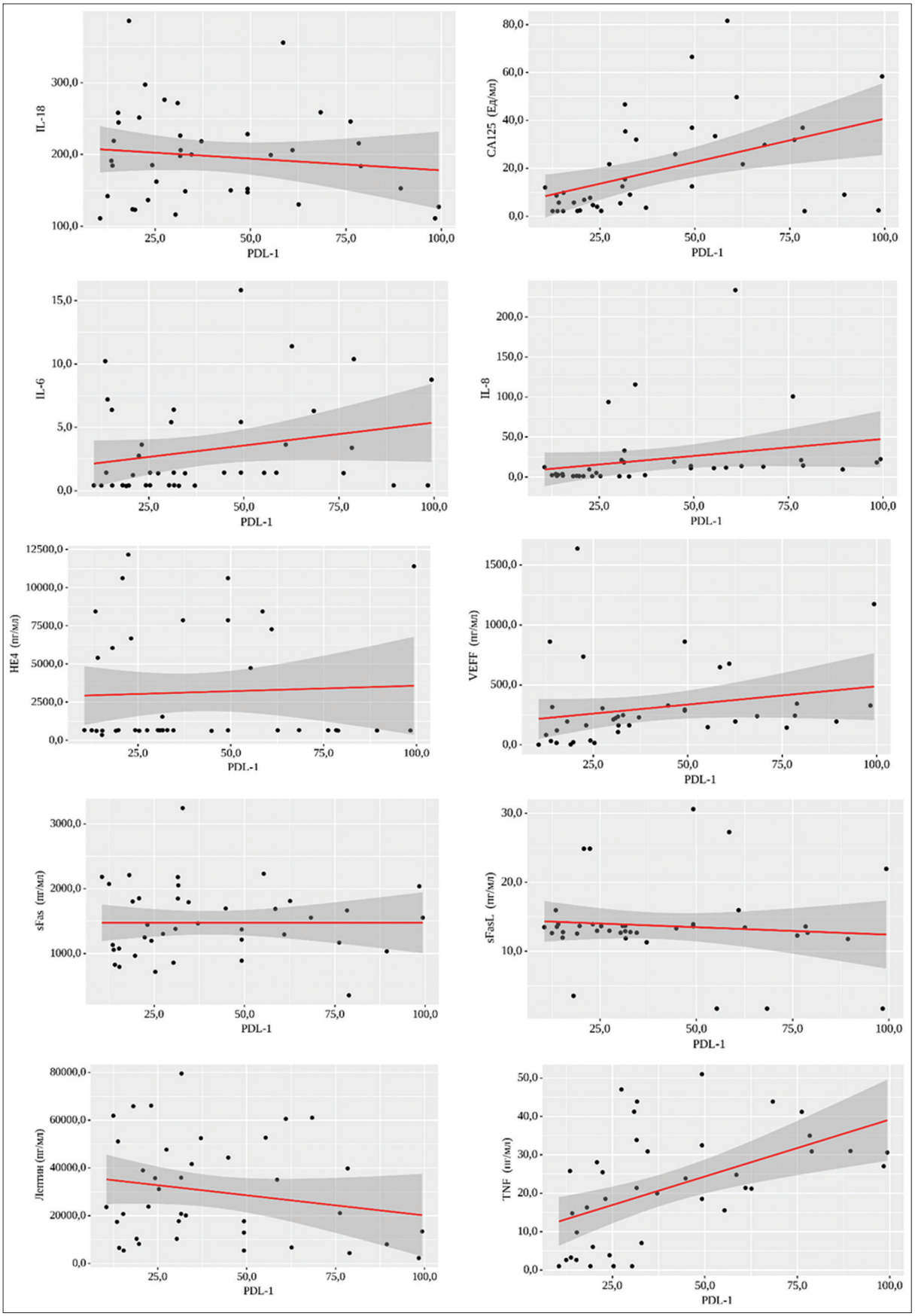

Figure 1: The regression function characterizing the dependence of the level of the studied diagnostic markers on the ligand programmed death-1 level

Table 2: Analysis of the studied diagnostic markers depending on the groups

Indicators	Category	Group_1			p
		Me	Q_1–Q_3	n	
PDL-1 (pg/ml)	Control group	19.72	14.72–24.78	19	<0.001*
	Pilot group	55.32	34.53–76.20	21	
IL-18 (pg/ml)	Control group	184.55	139.34–231.78	19	0.440
	Pilot group	200.07	152.16–228.41	21	
CA125 (unit/ml)	Control group	4.68	2.22–7.28	19	<0.001*
	Pilot group	31.87	15.43–36.96	21	
HE4 (pg/ml)	Control group	669.10	633.49–6361.22	19	0.892
	Pilot group	662.80	657.70–4731.30	21	
IL-6 (pg/ml)	Control group	0.43	0.41–2.09	19	0.013*
	Pilot group	1.43	1.39–6.30	21	
IL-8 (pg/ml)	Control group	1.60	1.25–3.35	19	<0.001*
	Pilot group	18.42	13.90–22.42	21	
Leptin (pg/ml)	Control group	23816.43	13893.24–45057.24	19	0.797
	Pilot group	21096.29	12943.48–44342.15	21	
TNF (pg/ml)	Control group	6.04	1.83–17.42	19	<0.001*
	Pilot group	30.90	23.87–41.20	21	
VEFF (pg/ml)	Control group	119.86	18.66–238.15	19	0.016*
	Pilot group	243.44	194.56–328.07	21	
sFas (pg/ml)	Control group	1195.98	910.95–1826.99	19	0.228
	Pilot group	1553.20	1293.63–1810.13	21	
sFasL (pg/ml)	Control group	13.49	12.66–13.79	19	0.786
	Pilot group	13.32	12.29–13.69	21	

*Differences in indicators are statistically significant (p < 0.05), PDL: Ligand programmed death, CA: Cancer antigen 125, IL: Interleukin, TNF: Tumor necrosis factor, VEGF: Vascular endothelial growth factor.

A number of studies have shown the presence of human HE-4 hyperexpression in some malignant tumors, in particular ovarian cancer, endometrial cancer, and endometriosis. However, Mckinnon *et al.* demonstrated a significant increase in HE-4 and CA125 levels in endometriosis patients and a significant decrease to below control levels after treatment with combined oral contraceptives, continuous progestogens, and GnRH agonists compared to CA125 [16]. In the given study, HE-4 levels did not statistically differ when comparing the experimental and control groups.

Table 3: Analysis of PDL-1, CA 125, IL-6, IL-8, TNF, and VEFF markers depending on the studied diseases

Indicators	Category	Endometriosis according to ICD 10			p
		Me	Q_1–Q_3	n	
PDL-1 (pg/ml)	Adenomyosis	61.80	39.73–78.22	10	0.481
	Ovarian endometriosis	49.22	38.23–63.45	11	
CA125 (unit/ml)	Adenomyosis	21.74	9.85–31.96	10	0.029*
	Ovarian endometriosis	36.96	27.84–52.54	11	
IL-6 (pg/ml)	Adenomyosis	1.40	1.38–4.97	10	0.180
	Ovarian endometriosis	3.39	1.43–6.35	11	
IL-8 (pg/ml)	Adenomyosis	19.86	14.07–98.94	10	0.324
	Ovarian endometriosis	18.27	13.54–20.11	11	
TNF (pg/ml)	Adenomyosis	30.90	22.80–38.65	10	0.597
	Ovarian endometriosis	32.49	24.34–39.43	11	
VEFF (pg/ml)	Adenomyosis	208.09	170.74–322.39	10	0.324
	Ovarian endometriosis	285.37	237.35–487.82	11	

*Differences in indicators are statistically significant (p < 0.05), PDL: Ligand programmed death, CA: Cancer antigen 125, IL: Interleukin, TNF: Tumor necrosis factor, VEGF: Vascular endothelial growth factor.

The source of VEGF in women with endometriosis is macrophages. It is known that VEGF is responsible for angiogenesis in the tissues of the endometrium, which allows it to recover after menstruation. It also affects the newly formed vessels. The studies conducted by Xavier *et al.* on 25 patients with endometriosis (Stages III–IV according to ASRM) and 13 patients in a control group, observed an increase in the VEGF levels during the secretory phase of the menstrual cycle and TNF-α in all phases of the cycle [17]. According to our results, the differences in the levels of TNF-α and VEGF in the experimental group are statistically significant ($p < 0.001$ and $p = 0.016$, respectively) compared to the control group.

Table 4: Results of correlation analysis of the relationship between the PDL-1 marker and other diagnostic markers under the study

Indicators	Correlation characteristics		
	p	Connection closeness as per the Cheddock scale	p
PDL-1–IL-18	-0.046	No connection	0.778
PDL-1–CA125	0.538	Noticeable	<0.001*
PDL-1–HE4	0.080	No connection	0.623
PDL-1–IL-6	0.248	Weak	0.123
PDL-1–IL-8	0.576	Noticeable	<0.001*
PDL-1–leptin	-0.144	Weak	0.374
PDL-1–TNF	0.581	Noticeable	<0.001*
PDL-1–VEFF	0.426	Moderate	0.006*
PDL-1–sFas	0.076	No connection	0.641
PDL-1–sFasL	-0.131	Weak	0.421

*Differences in indicators are statistically significant (p < 0.05), PDL: Ligand programmed death, CA: Cancer antigen 125, IL: Interleukin, TNF: Tumor necrosis factor, VEGF: Vascular endothelial growth factor.

The FAS-FASL system is one of the pathways of apoptosis, which plays a key role in the process of endometrial apoptosis. Sbracia *et al.* assessed FAS-FASL expression in the tissue samples obtained from 33 women with severe endometriosis and 18 women without endometriosis. Immunostaining for Fas ligand in the eutopic endometrium was stronger in the epithelial cells of the secretory phase, while epithelial cells of endometrioid lesions showed significantly stronger staining for the Fas ligand independent of the menstrual phase ($p < 0.01$). Immunostaining for FAS in the eutopic endometrium showed reduced staining during the proliferative phase, whereas it was strong in the secretory phase. The Fas reduced expression in the ectopic endometrium with modern higher expression of Fas ligand in the corresponding cells suggests a possible immune privilege of this tissue [18]. Our study found no significant differences in the sFAS and sFASL levels.

The statistical power of the given study is limited (only 21 patients in the experimental group). To the best of our knowledge, this is the first study to evaluate the concentration relationships of the PDL-1 markers, sFas, FasL, VEGF, ILs (IL-6, IL-8, and IL-18), CA125, HE4, and serum leptin in the patients with genital endometriosis.

Conclusions

This pilot study revealed the role of PDL-1 cells in genital endometriosis. The results of the study demonstrated that the levels of PDL-1, CA 125, IL-6, IL-8, TNF, and VEFF were statistically significantly different in the experimental and control groups. The correlation of PDL-1 levels with CA125, IL-8, and TNF in the patients with genital endometriosis was also revealed. The studies of PDL-1 with immunological markers of inflammation may open up new therapeutic strategies for the treatment of genital endometriosis in the future.

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